Developmental morphology of a lyriform organ in the Western black widow (*Latrodectus hesperus*)

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Keywords: *Latrodectus hesperus*, lyriform organ, slit sensilla, development, allometry, vibration detection.

Abstract

For most spiders their sensory world is dominated by their ability to detect vibrational stimuli. The organs responsible for detecting substrate vibrations are located on the animals' extremities and known as lyriform organs; close aggregations of membrane-covered slits in the cuticular exoskeleton. The morphology and geometry of the lyriform organ is an important determinant of how it functions and the range of stimuli it can detect. Most work on the morphology, mechanics, and physiology of lyriform organs has been conducted on adult wandering spiders, *Cupiennius salei,* and little is known about the morphology in other species or juveniles. We examine the morphology of the HS10 lyriform organ in both adult and juvenile Western black widows (*Latrodectus hesperus*). We find hypoallometric scaling of the lyriform organ, and the size of individual slits when compared to body size. However, the cuticular pad distal to HS10 scales isometrically across successive instars. We also find an increase in the number of slits within the lyriform organ with each moult. Future work should address physiological responses of the organ across development, which could lead to a better understanding of the function of the cuticular pad and stimuli pertinent to the survival of little studied juvenile spiders.

Introduction

Spiders are exquisitely sensitive to vibration. From species that dwell and hunt on plants and the ground to those that use silk webs and traps, vibration is commonly the stimulus modality used to detect prey (Masters and Markl 1981; Klärner and Barth 1982; Landolfa and Barth 1996), predators (Uetz et al. 2002; Lohrey et al. 2009; Uma and Weiss 2012) and potential mates (Schüch and Barth 1985; Maklakov et al. 2003; Elias 2003; Elias et al. 2005; Vibert et al. 2014). Vibrational signals are detected with mechanoreceptive strain sensors embedded within the cuticular exoskeleton (see Barth 2004; Barth 2012 for reviews). Known as slit sensilla, these sensors detect strain in the cuticle, and can be found singly, in loose clusters, or as distinct groupings known as lyriform organs. Their function spans proprioreception of self-generated strains from muscle contraction and haemolymph pressure changes, to detection of externally generated vibrations (Barth 2012). However, it is the lyriform organs that are primarily concerned with detecting vibrational displacement from the external environment; stimuli that can be in the order of mere nanometres (Barth and Geethabali 1982; Hössl et al. 2009).

Spiders have many lyriform organs which are exclusively found on their extremities, most frequently at joints on distal sections of the legs (Pringle 1955; Barth 1971; Barth and Stagl 1976). The organs are

composed of an array of membrane-covered slits in the cuticular exoskeleton. When a vibrational stimulus is transmitted to the lyriform organ, the compliant membranes are compressed by the surrounding hard cuticle which stimulates neural dendrites (Walcott 1969; Barth et al. 1984; Molina et al. 2009). Each slit within an organ has its own sensory cells and responds independently to a stimulus (Barth and Geethabali 1982). The attachment of the dendrite to the outer membrane is visible externally and is known as the coupling cylinder, the position of which does not necessarily correspond to the position of maximal compression of the slit (Hössl et al. 2007).

The main lyriform organ involved in detecting substrate vibrations is the HS10 organ, found dorsally on the distal end of the metatarsus (MT), just proximal of the metatarsal-tarsal (MT-T) joint (Barth and Libera 1970). Substrate vibrations are transmitted up the tarsus (T) causing it to strike the MT, at the MT-T joint. At the distal end of the metatarsus, between the HS10 organ and MT-T joint, resides a cuticular pad. In the wandering spider, *Cupiennius salei*, this pad is heterogeneous in composition, being soft and compliant at its distal end, but hard and sclerotized at the proximal end, adjacent to the organ (Young et al. 2014; Erko et al. 2015). The pad acts as a high pass filter, reducing the transmission of biologically irrelevant stimuli below 40Hz, but also protecting the organ from high amplitude stimuli that are potentially damaging (McConney et al. 2007; Young et al. 2014). The location of the slit within the lyriform organ, its length and its aspect ratio are all important factors in determining sensitivity to stimuli (Hössl et al. 2006; Hössl et al. 2007; Hössl et al. 2009), and with its own filter, the spider has evolved to detect a specific range of stimuli relevant to its survival and reproduction. Morphological aspects of the lyriform organ are crucial to its function, raising the question of how lyriform organs retain their function throughout development where the size of the animal changes significantly.

Much of what has been learned about the physiology, mechanics and morphology of lyriform organs has come from work on the wandering spider, *Cupiennius salei*, (see Barth 2012 for review) although some work has also been conducted on web dwelling species (Finck 1981; Klärner and Barth 1982; Barth 2002). The slit arrangement in HS10 in 4 spider species share a ‘basic pattern’ but variable numbers of slits; from 21 slits in the *C. salei* to just 8-10 in the American house spider *Achaearanea tepidariorum*. In addition to this the length of slits 2 and 11 in the HS10 organ have been measured for 9 spider species (Barth 2002). Although there are comparisons of slit numbers in lyriform organs between different instars in *C. salei* (Barth and Libera 1970), little is known about the development of this organ throughout a spider’s life span, or indeed to vibrational senses in juveniles in general.

We examine the HS10 metatarsal lyriform organ of the Western black widow, *Latrodectus hesperus*, throughout developing instars to adult spiders (Figure 1). *L. hesperus* is a web-dwelling spider that builds cob webs in arid environments (Kaston 1970). After emerging from the egg sac, spiderlings spend up to 14 days in the natal web, before dispersing and remaining on solitary webs for the majority of their lives (Kaston 1970). Mature males abandon their solitary webs to seek out female mates where they perform courtship displays with a vibratory component (Ross and Smith 1979; Kasumovic and Andrade 2004; Vibert et al. 2014). These courtship vibrations are not a stimulus found in juvenile environments. However, the need to detect prey and predators will persist throughout all life stages. Because the sensitivity of lyriform organs depends on their morphology, it is imperative to understand how they change throughout spider development. Developmental changes in morphology are likely to impact the range of environmental stimuli each instar is able to perceive; we would therefore expect minimal changes to overall organ morphology if the biologically relevant stimuli within the environment remain consistent throughout different stages of growth.

Materials and Methods

L. hesperus egg sacs were collected and incubated in plastic containers (length x width x height cm^3 = 8.73 x 8.73 x 11.27; Amac Plastics) at 25°C until spiderlings emerged. In keeping with previous terminology, the first instar was defined as the instar that emerged from the egg sac (Kaston 1970). Shortly after emergence, spiderlings were transferred to individual containers (length x width x height (cm) = 4.13 x 4.13 x 5.56) where they were allowed to moult into successive instars at 24°C. Up to 20 spiderlings were taken from each instar group, and euthanised in 70% ethanol where they were kept until imaging.

Images of the HS10 metatarsal lyriform organ were taken using scanning electron microscopy (SEM). To prepare samples for imaging spiders were removed from ethanol and affixed to an SEM stub using double sided tape. For early instars the entire animal was positioned on the stub, but for adults and later instars the first pair of legs were removed using fine scissors and positioned on the stub. Photographs were taken with a Nikon DXM 1200 camera mounted on a Zeiss Stemi 2000-C dissecting microscope. Images of the first pair of legs were taken, using Act-1 (Nikon Corp. 2000; Figure 1C), to allow measurement of dimensions of the MT. Where necessary, hairs were shaved from around the HS10 organ, however this was often not possible on smaller animals without causing damage to the surrounding tissue or leaving behind significant debris. Samples were subsequently sputter coated with gold (PS3, Polaron, Watford, UK) before SEM imaging (Hitachi S530 SEM, Hitachi, Tokyo, Japan). Images were acquired and digitised using Quartz PCI imaging software (Quartz PCI, Quartz Imaging

Corporation, Vancouver, Canada) and morphological measurements made using Corel Draw (Corel Corporation, Ottawa, Canada) and the JH CurveLength macro.

Results

The HS10 organ is present from the first instar through to adulthood in *L. hesperus*. There is a basic set of 13 slits that are present across all instars examined (Figure 2 and 3). At each moult between instars 1-5, HS10 gains an additional slit, added to the proximal end of the HS10 organ. The maximum number of slits seen in any metatarsal HS10 organ was 23, and this number was only seen on the largest spiders, the adult females. Males had a maximum of 20 slits. It was only possible to measure the first 5 instars due to mortality so it is not possible to tell whether the addition of a single slit at each moult remains the case after the 5^{th} instar into the final moult to adulthood. The positions of the slits are largely conserved between individuals, although there is a small degree of variation (Figure 3).

The coupling cylinder was visible in the larger HS10's of the adults due to the greater slit width. It was also possible to see it in the SEM images of at least some juveniles in instars 1-4 (Figure 4). The position of the coupling cylinder in adults of both sexes is somewhat offset from the centre of the longitudinal length of the slit, corresponding with previous findings in the wandering spider *C. salei* (Barth and Pickelmann 1975).

Relative to the growth of the MT, the HS10 organ shows little change in size across development and has a hypoallometric relationship with MT length. The allometric coefficient (α), or slope of a straight line fit to the log data is less than 1 (α = 0.45, figure 5a). Slit length is also hypoallometric throughout development, with a slightly smaller allometric coefficient (α = 0.31-0.36, figure 5b). To reach mean adult male size, MT increases 7.9 times. This is a far greater rate than the 2.6-2.9 times increase in slit length to reach adult female size (1.4-1.9 fold increase to full adult male size) and HS10 width which grows slightly more than slit length to 3.2 times the size of the first instar in adult females, and 1.8 times for adult males. In contrast, the cuticular pad at the distal end of the MT develops isometrically with MT length (α = 0.99, figure 5). Using MT length as a proxy, first instar spiders increase in size 12.7 times to reach the mean size of an adult female. The pad is pronounced in adults of both sexes, but in the 5 early instars measured, it is small in size (Figure 3).

Discussion

The slit arrangement in the HS10 organ of *L. hesperus* resembles those in other web building species, in particular *Zygiella x-notata* (Barth 2002). Similarly, the location of dendritic attachment to the outer membrane, visible externally as the coupling cylinder (Figure 4), does not significantly deviate from the basic pattern found in other species previeously investigated (Barth 2002). It is possible that the number of slits present in the HS10 organ of *L. hesperus* reflects the number of moults that the animal has undergone. Kaston (1970) observed female *L. hesperus* moulting 9 times, occasionally up to 10, while males can mature twice as fast moulting up to 7 times. We observed the addition of a single slit per moult up to the 5th instar. If this trend continues to the final moult, then these females would have undergone a maximum of 10 moults (23 slits, with first instars having the basic 13 slits), and males 7 (20 slits), which is in fact what we observed. Ecological conditions can influence the rate of development in spiders, and the number of instars before the final moult. If it is the case that the number of slits in the HS10 lyriform organ corresponds to the number of moults, as seems likely from our results, it may be possible to use this as a tool in further studies where adults are available and the number of moults are of interest in developmental studies.

The aspect ratio of the slits within a lyriform organ determine its sensitivity to vibrational stimuli, with smaller slits requiring higher loads to generate enough deformation for a nervous response (Hössl et al. 2006; Hössl et al. 2007; Hössl et al. 2009). Allometry is defined as differential growth rates of body parts (Huxley et al. 1941). With the importance of morphology on the function of the lyriform organ it is perhaps unsurprising that HS10 develops hypoallometrically (slower rate than body size) compared with body size. However, it is somewhat surprising that the cuticular pad, an important filter physically protecting the lyriform organ from high load stimuli, in contrast to HS10, grows isometrically (same rate as body size). If the need to filter stimuli remains consistent from juvenile to adult then it is curious that the pad should not scale hypoallometrically, like the HS10 organ, to minimise changes in its filter properties.

The metatarsal pad distal of the HS10 lyriform organ in *L. hesperus* is small relative to that found in the wandering spider *Cupiennius salei,* the subject of previous studies on this structure (McConney et al. 2007; Young et al. 2014; Erko et al. 2015). In all but adult *L. hesperus* there is a very small area of cuticle distal to HS10. In male and females this becomes a larger structure that resembles the 'appendix' area found in *Cupiennius* (Erko et al. 2015). In *Cupiennius* the metatarsal pad acts as a high-pass filter, removing biologically irrelevant stimuli below 40Hz (McConney et al. 2007), its material properties being complex and heterogeneous to perform this task (Young et al. 2014; Erko et al. 2015). Unlike the distal portion of the pad which is soft and compliant, the 'appendix' adjacent to slit 1 of HS10 is hard and sclerotized (Erko et al. 2015). Without analysis of the material properties of the pad

in adults, or indeed juvenile *L. hesperus,* it is not possible to infer whether their pad performs the same function as in *Cupiennius* or whether it has a different structure specific to the web substrate that transmits vibrational signals in *L. hesperus*. The angle of the joint on which the lyriform organ is located also affects responses to vibrational stimuli (Finck 1981). The T of *Cupiennius* has a wider range of angular motion before cuticular structures of the MT and T come into contact than that of the web-dwelling species, *Nephila clavipes* (Barth 2002; Schaber et al. 2012). L*. hesperus* and *Cupiennius* inhabit different substrate types, and show considerable differences in the angle of MT-T joint superficially appearing similar to those observed in *Nephila*, which could reflect differences in pad structure and function.

The lack of any significant pad in juvenile spiders may imply that they do not require much signal filtering in order to respond to relevant stimuli, but there are also other explanations that are not mutually exclusive. As well as acting as a filter it is thought that the pad serves to protect the HS10 organ from high amplitude, potentially damaging stimuli (Erko et al. 2015). This function is perhaps more relevant to adults than juveniles, as in juveniles a damaged structure will be replaced with a fully functioning new structure at the next moult. Cumulative damage to HS10 should therefore be more of a problem in adults and could be an explanation for the greater change in morphology over development of the pad relative to the lyriform organ itself.

Small spiders need to be able to detect prey and potential predators as much as far larger adult spiders, and although behaviour and the overall composition of prey and predators may differ somewhat between small spiders and large adults, there is overlap and detection of similar stimuli will be required to survive (see Uma and Weiss 2012). However, very little is known about the ecology of juvenile *L. hesperus* or indeed juvenile spiders in general. Further work should include electrophysiological measurements of spiderling lyriform organs in response to vibrational stimuli in order to determine whether the sensitivity of the organ changes throughout the development of the animal. This would also give insight into the function of the cuticular pad through comparison of responses between juveniles and adults whose pad sizes differ far more than the size of the lyriform organ.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Human and animal rights

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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291 10.1016/j.actbio.2014.07.023

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Figure Legends

Fig. 1 A) Adult female *L. hesperus* on her web. B) Metatarsal lyriform organ (HS10) measured is located on the dorsal side of the metatarsus at the metatarsus - tarsus joint (arrows). C) First pair of legs of a second instar *L. hesperus*. Arrows indicate metatarsus length. MT = metatarsus, T = tarsus

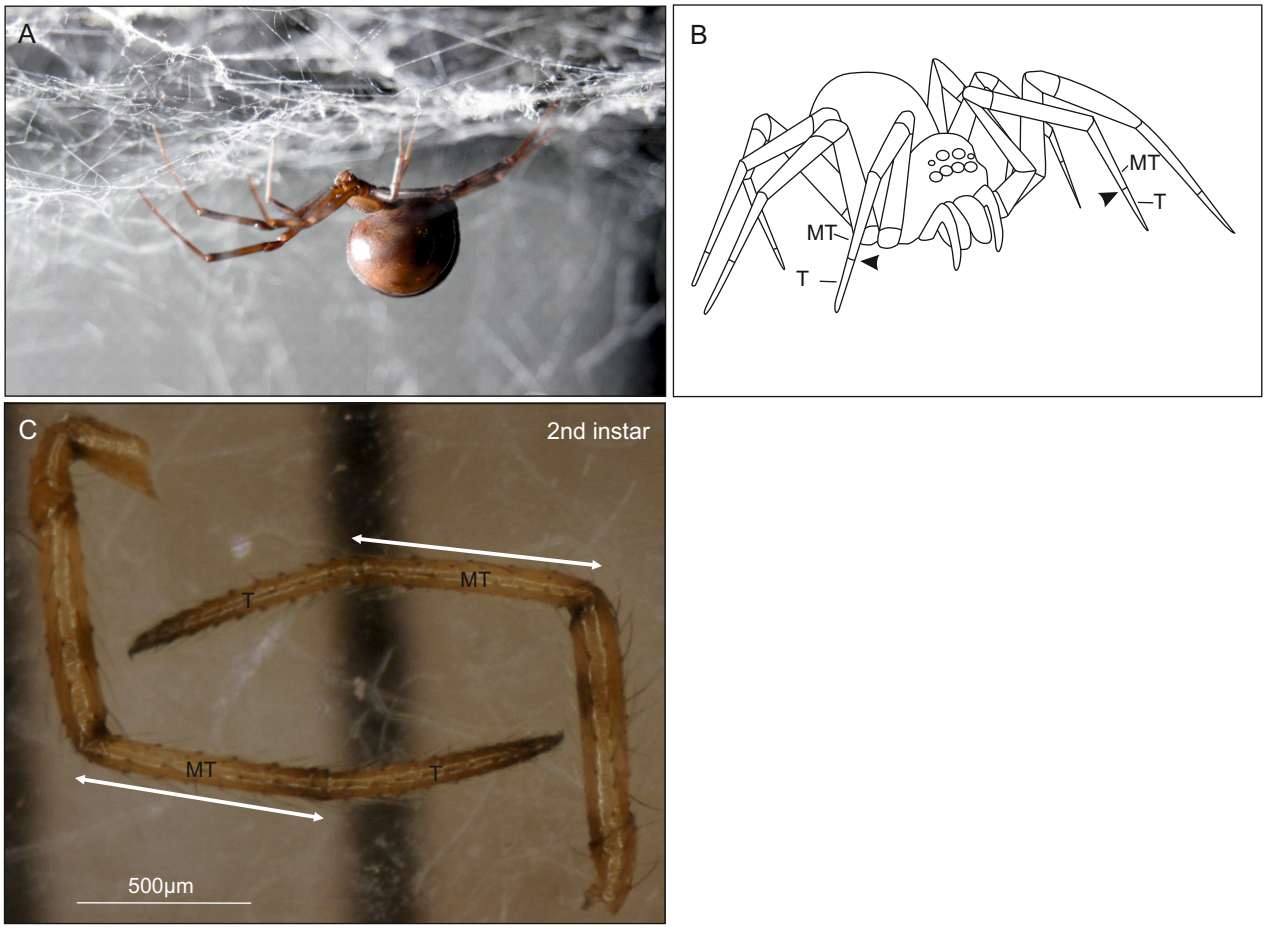
Fig. 2 SEM images of the black widow (*L. hesperus*) HS10 lyriform organ. A) first instar; B) second instar; C) third instar; D) fourth instar; E) fifth instar; F) adult male; G) adult female; H) slit length for slit 2 (grey) and 11 (white) in adult male and females. N= 7.

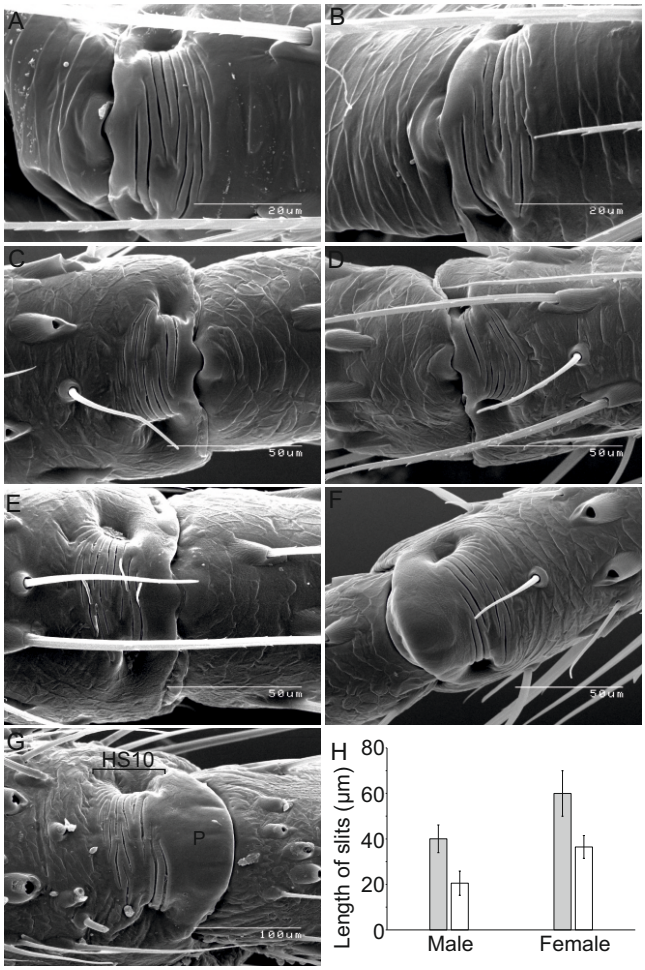
Fig. 3 External morphology of the lyriform organ across all instars illustrated at the same scale. Adult female slits are numbered

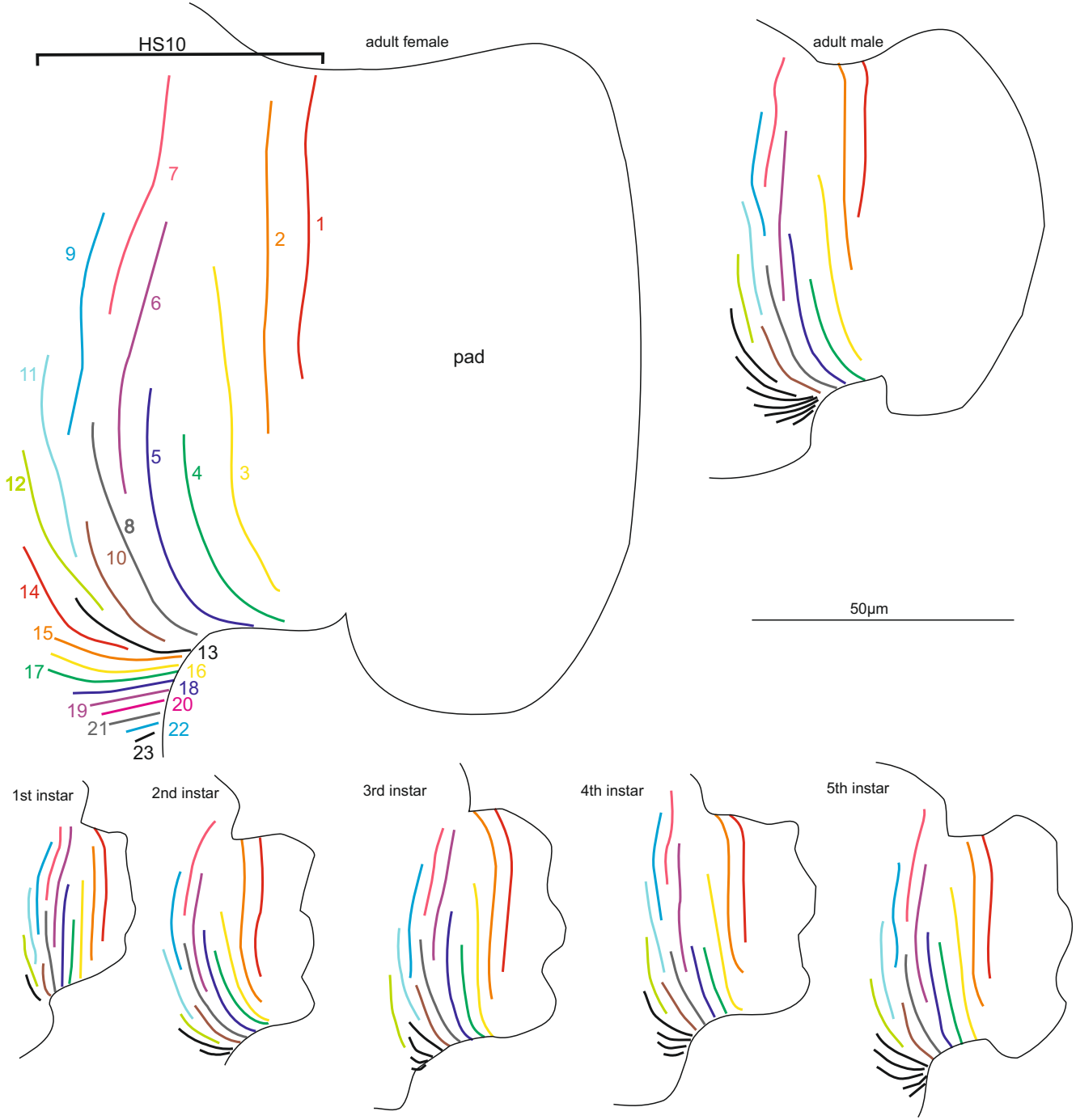
Fig. 4 Position of coupling cylinder in HS10. A, B, C and D are first to fourth instar, respectively. E) Adult male F) Adult female. No specimens had all coupling cylinders visible simultaneously, especially in younger instars. Arrows indicate position of coupling cylinder. P indicates position of pad

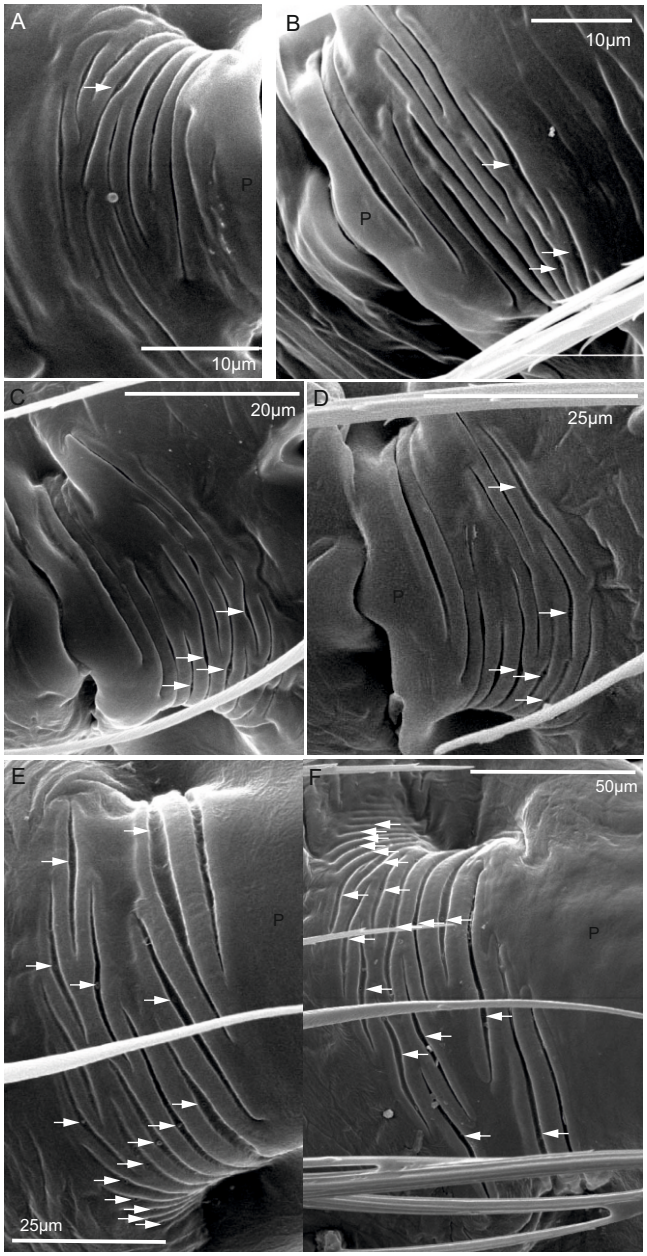
Fig. 5 A) Log MT length against log HS10 width (black) and pad width (hollow). Straight line fits to the data show isometric growth of the pad with MT (dashed) and hypoallometric growth of the HS10 (black). B) Log MT length against log slit length and pad width (hollow circles). Straight line fits to the data show isometric growth of the pad with MT (dashed) and hypoallometric growth of slit 1 (grey squares), slit 2 (white squares) and slit 9 (black squares)

Table 1 Measurements from the MT and HS10 across all instars and both adult sexes measured. Width of pad was measured between its distal margin and slit 1. Width of HS10 is measured between slit 1 and 12. MT length is measured along its dorsal edge.

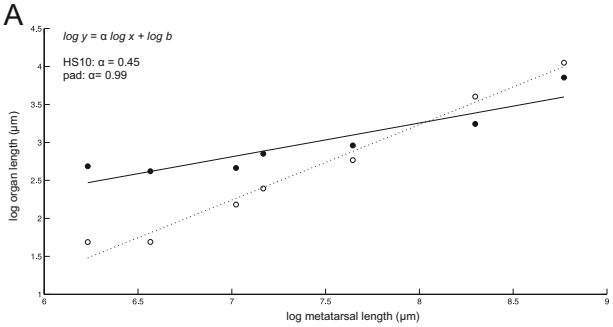

A
B
MT
T
MT
T
C
2nd instar
T
MT
MT
T
500µm


A
20um
B
20um
C
50um
D
50um
E
50um
F
50um
G
HS10
P
100um
H
Length of slits (µm)
80
60
40
20
0
Male
Female

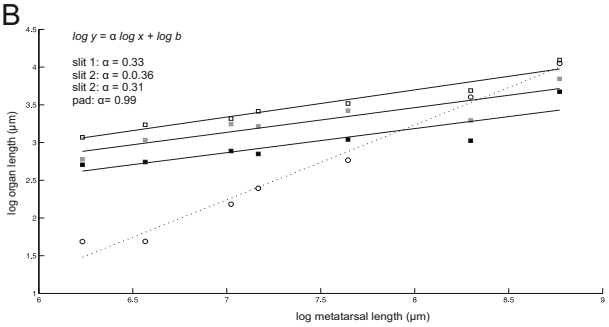

HS10
adult female
adult male
pad
50µm
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
1st instar
2nd instar
3rd instar
4th instar
5th instar


A
P
10µm
B
10µm
P
C
20µm
D
25µm
P
E
P
25µm
F
50µm
P

A


log y = α log x + log b
HS10: α = 0.45
pad: α= 0.99
log organ length (µm)
log metatarsal length (µm)
4.5
4
3.5
3
2.5
2
1.5
1
6
6.5
7
7.5
8
8.5
9

B


log y = α log x + log b
slit 1: α = 0.33
slit 2: α = 0.0.36
slit 2: α = 0.31
pad: α= 0.99
log organ length (µm)
log metatarsal length (µm)
4.5
4
3.5
3
2.5
2
1.5
1
6
6.5
7
7.5
8
8.5
9

Instar	MT length (mm)	Pad width (µm)	HS10 width (µm)	Max. slits seen
1st	0.51 ± 0.02 (n=10)	5.4 ± 0.25 (n=6)	14.7 ± 1.56 (n=6)	13 (n=6)
2nd	0.71 ± 0.05 (n=10)	5.4 ± 2.12 (n=9)	13.7 ± 2.12(n=9)	14 (n=10)
3rd	1.12 ± 0.03 (n=10)	8.9 ± 1.25 (n=7)	18.1 ± 1.39 (n=7)	15 (n=7)
4th	1.30 ± 0.17 (n=4)	11.0 ± 2.80 (n=4)	17.3 ± 2.15 (n=3)	16 (n=3)
5th	2.09 ± 0.08 (n=2)	15.9 (n=1)	22.9 (n=1)	16 (n=1)
Adult (m)	4.02 ± 0.08 (n=9)	37.2 ± 5.64 (n=9)	26.19 ± 3.30 (n=9)	20 (n=9)
Adult (f)	6.44 ± 0.32 (n=10)	57.4 ± 11.16 (n=10)	47.17 ± 6.63 (n=10)	23 (n=9)